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Spatial summation of blue-on-yellow light increments and decrements in human vision

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Abstract

In the primate retina, blue-OFF cells are less numerous than blue-ON cells but no psychophysical equivalent of this asymmetry has been found so far. The hypothesis put forward in the present study is that the ON–OFF asymmetry should manifest itself in the size and effectiveness of spatial summation of S-cone signals of opposite polarity. To test this hypothesis upon selective stimulation of the S-cones in man, a 3 cd/m^2 blue light was superimposed on a 300 cd/m^2 yellow background and the test stimulus consisted in a luminance increment or decrement of the blue light from its steady level over a circular area of variable size. The test stimuli were presented at 12.5° retinal eccentricity. Within the test-stimulus spectral band, sensitivity was that of Stiles' π_1 mechanism. Increasing stimulus area reduced more the decrement threshold than the increment threshold, and Ricco's area was larger for luminance decrements (0.8–2°) than for increments (0.6–0.9°). Experiments with red-on-red stimuli confirmed that the large summation area and stimulus-polarity-dependent spatial summation are specific for the isolated S-cone signals. The sign-dependency of spatial summation is probably a psychophysical correlate of the asymmetry of the ON- and OFF- visual pathways receiving S-cone input.

Keywords: S-cones; Spatial summation; Increment threshold; Decrement threshold; ON/OFF-pathways

1. Introduction

The short-wavelength sensitive cones (S-cones) are the least numerous in the primate retina yet they 'have received a disproportionate amount of attention' (Curcio et al., 1991). There are both theoretical and practical reasons for this attention. Of theoretical interest are such questions as of the spatial and temporal resolution of the S-cone pathway in comparison with the M- and L-cone related pathways (e.g. Green, 1968; Brindley, 1970; Mollon, 1982; Mullen, 1990) as well as of whether S-cones contribute to the luminance channel (Stockman, MacLeod & DePriest, 1991). Of practical interest are the findings of blue-yellow colour vision deficits (Sample, Weinreb & Boynton, 1986) and blue-on-yellow sensitivity loss (Casson, Johnson & Shapiro, 1993) that may have some predictive value in determining which ocular hypertensives are at risk of developing glaucoma as well as in monitoring the visual field loss in glaucoma patients.

The S-cone pathways differ from the other afferent visual pathways in several respects and one of them is the ON–OFF asymmetry. The retinal ganglion cells receiving an S-cone input are asymmetrically divided between the ON and OFF classes. The density of the 'blue-OFF cells' is estimated to be about twice as low as that of the 'blue-ON cells' (Lee, 1996) or even lower (Malpeli & Schiller, 1978; De Monasterio, 1979). The dissimilarity concerns in fact all cellular elements from cone bipolars (Mariani, 1984), to at least the lateral geniculate cells (Derrington, Krauskopf & Lennie, 1984; Valberg, Lee & Tigwell, 1986). These estimates are based on extracellular recording studies. The blue-

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ON ganglion cells have been identified as the recently discovered small-field bistratified cells (Dacey & Lee, 1994). No blue-OFF ganglion cells have been identified morphologically or in intracellular recordings and there are even doubts in their existence (Martin, 1998).

Several studies aimed at finding a psychophysical correlate of the blue ON–OFF asymmetry. They all tested the hypothesis that the lower density of OFF neurons in comparison with the ON neurons should result in a lower sensitivity to stimulus offset than onset. De Marco, Smith and Pokorny (1994) presented rapid-on and rapid-off sawtooth stimuli to selectively stimulate the ON or OFF pathways. Using long-wavelength chromatic adaptation, they did not find any bias for detection of rapid increments over rapid decrements for S-cone pathways. Schwartz (1996) obtained spectral sensitivity curves with test stimuli that were either isolated step onsets or offsets presented on an intense background (colour temperature 3300 K). The spectral sensitivity curves did not exhibit the expected reduction for blue offsets. Smith, Harwerth, Crawford and Duncan (1989) injected rhesus monkeys with 2-amino-4-phosphonobutiric acid (APB), a pharmacological agent that is believed to selectively reduce responsiveness of retinal ON neurons, and measured the spectral sensitivity before and after APB under both scotopic and photopic vision. While APB substantially reduced the rod-mediated spectral sensitivity, there was no significant change in cone-mediated sensitivity including sensitivity associated with the S-cones. The joint conclusion of all the above studies is that the ability of the OFF system to process S-cone signals is comparable with that of the ON system and, therefore, the psychophysical data are at variance with the physiological data suggesting paucity of the S-cone OFF neurons.

The present study was aimed at verifying the hypothesis that the S-cone ON–OFF asymmetry should reveal itself in the extent of spatial summation of S-cone signals for blue-light increments and decrements. Spatial summation can be defined as a convergence of signals from neighbouring points of the visual field onto a common pathway and it is usually assumed that the most likely, although not the only possible, site for such a convergence is within the retina (Brindley, 1970). Two psychophysical laws are commonly considered as related to spatial summation (e.g. Brindley, 1970). The first one is Ricco's law stating that the threshold luminance of a stimulus is inversely proportional to its area so that the threshold luminous flux is independent of its spatial distribution. The area up to which this law holds is known as Ricco's area. Outside Ricco's area spatial summation follows more or less precisely Piper's law according to which the threshold luminance of the stimulus is inversely proportional to the square root of its area. Ricco's area increases with retinal eccentricity (e.g. Wilson, 1970) and spatial resolution decreases with eccentricity in an inverse proportion to retinal ganglion cell density (Virsu, Rovamo, Laurinen & Näsänen, 1982). Morphological data suggest that all ganglion cell classes produce an efficient and economical nearly constant coverage of the retina with their dendritic fields (Wässle & Boycott, 1991).The less dense cellular types should have larger dendritic fields and indeed parasol cells are less dense and have larger dendritic fields than midged cells (Dacey & Petersen, 1992). As far as the receptive field central area is determined by the dendritic field size (Wässle & Boycott, 1991), the constant coverage implies an inverse proportionality between the receptive field central area and ganglion cell density (Virsu et al., 1982). If, in man, the densities of retinal blue-ON and blue-OFF cells differ as in other primates, summation of S-cone signals for light decrement should occur over a larger area than summation of S-cone signals for light increment.

2. Methods

A main experiment and two control experiments were performed. In the main experiment, either blue stimuli were presented on a background consisting of bright yellow and dim blue light or red stimuli were presented on a dim red background. Threshold luminance increment or decrement was measured as a function of the size of the test stimulus in order to obtain comparative data on the area of summation of luminance signals of opposite sign. The control experiments were aimed at: (a) verifying the isolation of the S-cones by the bright yellow background; and (b) checking the main findings for instrumental artefacts.

2.1. Main experiment

2.1.1. Apparatus

2.1.1.1. Blue-on-yellow stimuli. The two-colour threshold method of Stiles (Wyszecki & Stiles, 1982) was applied to isolate the S-cone signals. The background consisted of a 300 cd/m^2 yellow light component and a 3 cd/m^2 blue light component. (Due to individual differences in pupil size, the background retinal illumination by the yellow background component varied between 3400 and 5900 trolands.) The test stimulus consisted of blue luminance increment or decrement from the steady blue-component level over a circular area of variable diameter. The equipment arrangement is illustrated in Fig. 1. The light sources were a monitor (a 17 in. EIZO T562-T) controlled by a Visual Stimulus Generator (VSG 2/3 by Cambridge Research Systems) providing the blue component and a slide projector (Pentacon) providing the bright yellow component. Both sources were superimposed using a neutral

semitransparent mirror. The spectra of the monitor guns and the yellow background component were measured by means of a monochromator (Jobin Yvon) and a radiometer (Optronic 730A). The relative spectra of the monitor guns are shown in Fig. 2.

The blue gun of the monitor was the only one used in this part of the experiment. The distance from the monitor centre to the eye was 75 cm at which the screen covered an area of 21.7° horizontally and 16.5° vertically. Each pixel subtended 2×2 min of arc at the viewing distance. The light source in the slide projector was a halogen lamp. Its beam was passed through a yellow glass filter (PITMO, Russia-OC 12) and was

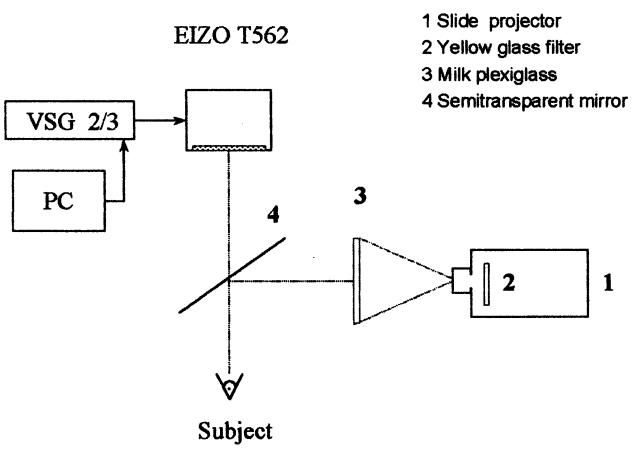

Fig. 1. Experimental set-up. A 17-inch EIZO T562 monitor was the source of the test stimuli. Stimulus colour, intensity, size and duration were controlled by a VSG 2/3 card hosted in a computer. This computer also controlled the experiment. The sources of the background field were the monitor and a slide projector. Both sources were superimposed at the eye by a semitransparent mirror (further explanations see in the text).

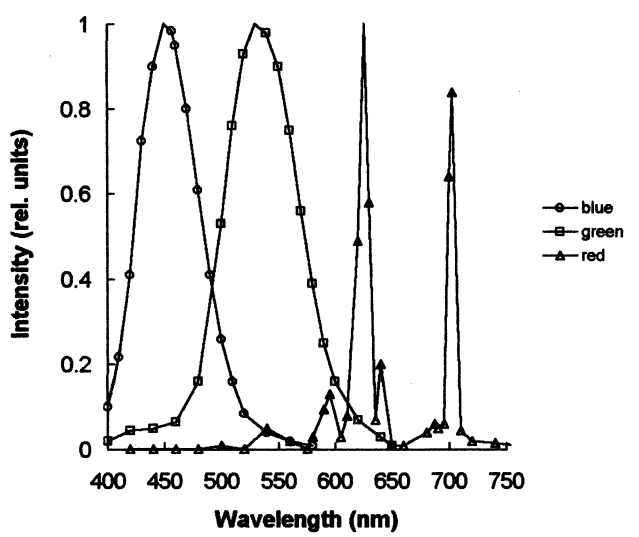

Fig. 2. Relative spectra of the blue, green and red guns of the monitor as measured in the present study. Data in units of the peak radiance for each gun.

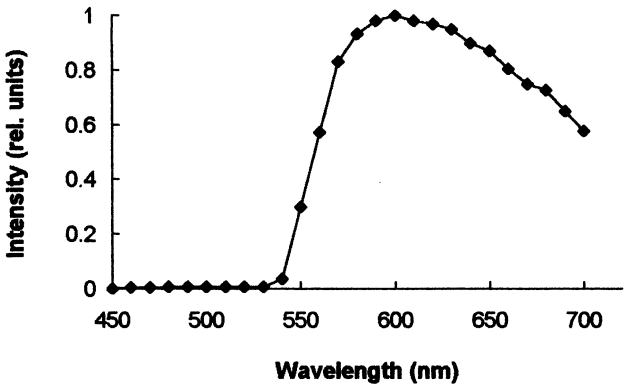

Fig. 3. Spectrum of the background component provided by the slide projector measured at the eye after the light has passed through the yellow filter (2 in Fig. 1) and has been reflected by the semitransparent mirror (4 in Fig. 1).

projected onto a milk-plexiglass so as to form a rectangle covering the monitor screen at the position of the eye. The spectrum of this light was measured at the eye position, i.e. after being reflected by the semitransparent mirror, and is shown in Fig. 3, light of wavelength above 540 nm with a maximum at 600 nm reached the eye.

Luminance was measured at the eye position by a Tektronix J6523 narrow-angle luminance probe connected to a Tektronix 716 Digital Photometer. Intensities were also measured by a Tektronix J6502. The blue background component was 170 mW/m^2 at the screen centre and the yellow component was 7700 mW/m^2 at the surface of the milk glass facing the subject. These values were approximately halved by the semitransparent mirror.

2.1.1.2. Red-on-red stimuli. In order to compare the data on spatial summation obtained upon the presumed S-cone isolation with data obtained upon stimulation of other than the S-cones, experiments were carried out during which both the background and the test stimulus were provided by the red gun of the EIZO monitor. Our measurements showed two narrow spectral bands for this gun well outside the S-cone sensitivity band (Fig. 2). Such light favours the L-cones rather than the M-cones (Stockman, MacLeod & Johnson, 1993). In this 'red-on-red experiment' we adjusted the red background at such a level as to obtain the same retinal illumination (in photopic trolands) as the illumination by the blue component of the background in the 'blue-on-yellow' experiment. To this end, the pupil size was to be taken into account in view of the different total background intensity resulting from the presence or absence of the yellow background component. Pupil size was measured at a number of luminance levels with each subject and the intensity of the red background that matched the blue component of the yellow-plus-

blue background in illuminating the retina was selected. The match should provide L-cone adaptation of the same level as the S-cone adaptation in the blue-on-yellow experiment. Spatial summation is known to depend on the adaptation state of the eye (Barlow, 1958; Glezer, 1965). Our approach relies on the assumption (see Krauskopf & Mollon, 1971) that adaptation is independent for each receptor type and is determined by the extent of light absorption by the photoreceptors.

The blue-on-yellow and red-on-red thresholds were measured alternatively on consecutive daily sessions.

2.1.1.3. Stimulus localisation. The present study is a part of a larger programme aimed at the possible invention of new methods for the diagnosis and follow-up of glaucoma. Therefore, following Tyler (1981), the test stimulus was positioned in a visual field area, specified below, which is assumed to be affected early in the development of glaucoma. In order to avoid screen luminance non-uniformity, the test stimulus was presented at the screen centre. The EIZO monitor provided a white cross at 7.5° below and 10° to the right of the screen centre to serve as a fixation mark and help accommodation. Viewing was with the left eye, thus the test stimulus appeared at 12.5° eccentricity in the upper temporal quadrant of the visual field of the eye.

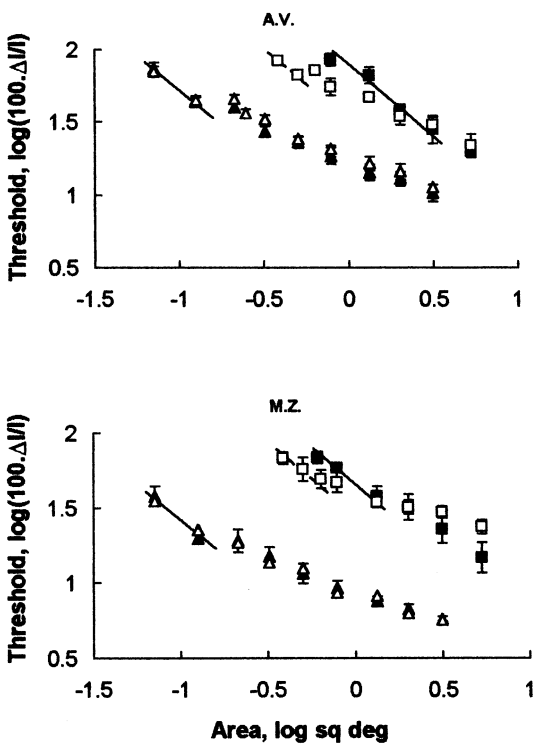

Fig. 4. Spatial summation of blue-on-yellow and red-on-red luminance increments and decrements. Squares — blue-on-yellow stimulation; triangles — red-on-red stimulation. Open symbols — luminance increment; closed symbols — luminance decrement. Vertical bars — the 95% confidence intervals of the mean. When not present, the confidence intervals are smaller than the symbols. Description of lines of the slope of −1 see in the text. Subjects A.V. and M.Z.

2.1.1.4. Viewing conditions. The stimuli were seen by the natural pupil. If necessary, refraction was corrected for the viewing distance of 75 cm. The visibility of separate horizontal lines of the screen upon either blue or red light was the criterion for correction of the refraction and corresponded to visual acuity of 0.97. (The frame frequency was 160 Hz at which the resolution was restricted to 480 horizontal lines or, at the viewing distance, to 29 lines/deg.) Given the above visual acuity, we assume that, within the range of stimulus sizes used, the accommodation error to blue light which is a common problem with blue stimuli, was not significant.

2.1.1.5. Stimulus duration. Stimulus duration was 100 ms. Within the intensity limits of our blue light source, up to 6 cd/m^2 transmitted through the mirror at the eye and a half of that as a mean level necessary to measure both increment and decrement thresholds, this was the shortest duration at which the threshold of small stimuli could be measured and the spatial summation area estimated. The rise and decay times within the test stimulus area were less than 1 ms as measured by a photodiode and an oscilloscope.

2.1.2. Psychophysical procedure

The psychophysical procedure used in most experiments was an yes/no double staircase method with 10% catch trials run by a computer. The independent variable was the magnitude of luminance increment or decrement from the steady level. The subjects started the trials by pressing a key and, 0.5 s later, a stimulus or a blank was presented. Two other keys were used by them to report whether the stimulus was seen or not. The false positive answers were less than five percent of the catch trials and, when given, were followed by an acoustic feedback. Any change in the type of response to the test stimulus led to a reversal in the staircase (1+ and 1− staircase variant, Levitt, 1971). The two staircases and the catch trials were randomly intermixed. At the start of each staircase, the step size was 0.2 log units and an adaptive procedure reduced it to 0.1 log units after the first reversal and to a final one of 0.05 log units after the second reversal. The procedure lasted until the accumulation of six reversals in each staircase at the 0.05 log units step and their geometrical mean was calculated as the threshold. Data from four to six sessions performed on different days were averaged as the final means.

In some experiments (Figs. 4 and 5), increment and decrement thresholds were measured in separate but interleaved daily sessions. The use of two parallel staircases was aimed at avoiding anticipation about stimulus visibility. In other experiments (Figs. 6 and 7),

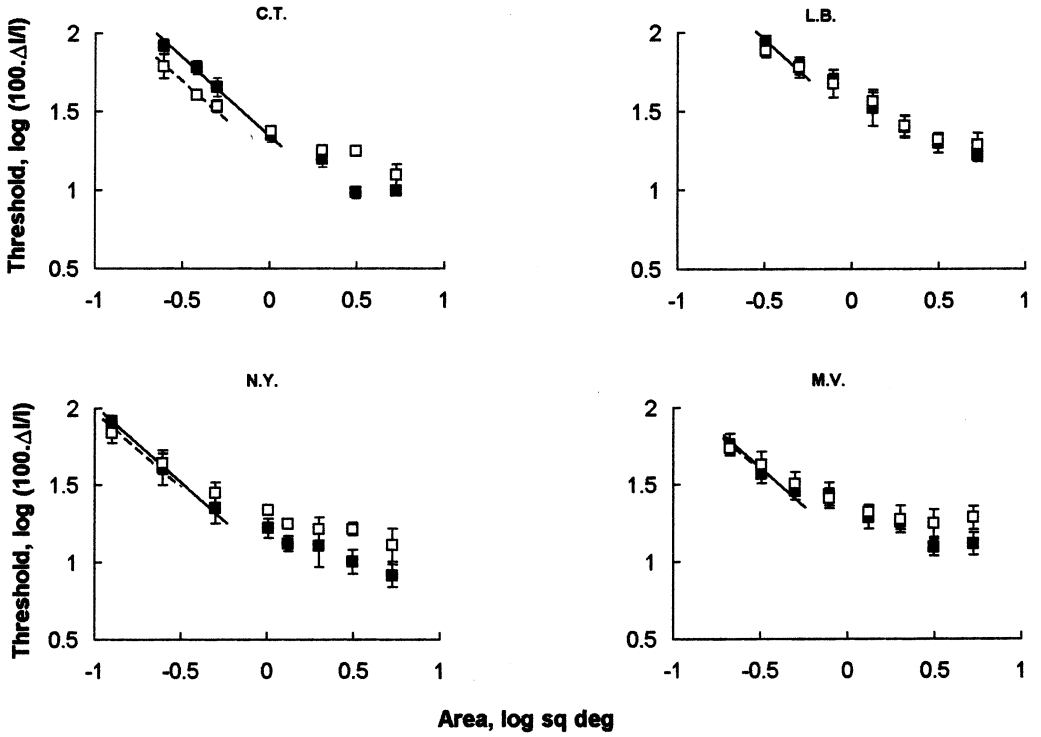

Fig. 5. Spatial summation of blue-on-yellow increments and decrements. Open symbols — luminance increment; closed symbols — luminance decrement. Vertical bars — the 95% confidence intervals of the means. Description of lines of the slope of −1 see in the text. Data of Subjects C.T., N.Y, M.V. and L.B.

both thresholds were measured within a block of trials with a single staircase for each type of threshold, the two staircases randomly intermixed.

2.2. Control experiments

2.2.1. Verification of the S-cone isolation

2.2.1.1. Spectral sensitivity. Spectral sensitivity was measured with the eye adapted to the 300 cd/m^2 yellow background. The source of the test stimulus light in this case was a Jobin Yvon monochromator set to provide spectral bands 8 nm wide. The monochromator took the place of the EIZO monitor in Fig. 1. A circular mask restricted the stimulus size to 2.1° in diameter. The mask covered a diffusing glass through which the light passed. A shutter switched on and off the light at the frequency of 1.25 Hz (400 ms stimulus pulses separated by 400 ms dark intervals). This frequency is within the range of maximum sensitivity of the blue mechanism (e.g. Budde, Korth & Mardin, 1997). Sensitivity was measured in 20 nm intervals in the 400–600 nm range.

The procedure of adjustment was applied to measure spectral sensitivity. The subjects adjusted the stimulus intensity at the threshold of visibility by neutral density filters and wedges taking as long time as needed. Stimulus intensity was measured after each setting by a J6502 Tektronix radiometer. Averages of six settings were obtained at each wavelength within two separate days.

2.2.1.2. Blue stimulus detectability at different luminance levels of the yellow background component. Both blue stimulus increment and decrement thresholds were measured simultaneously on blue plus yellow background with the blue component fixed at 3 cd/m^2, and the yellow component at different levels between zero and 520 cd/ m^2. The test stimulus consisted in luminance increment or decrement over a circular area, 1° in diameter, in the centre of the blue monitor screen. The fixation point was at the same location as in the main experiment.

2.2.2. Blue-on-blue stimulation

As it is seen in Section 3, significant differences between the increment and decrement thresholds were found upon blue-on-yellow stimulation. One might suggest that light scatter from areas of higher luminance on neighbouring areas of lower luminance would change differently the effective areas of test stimuli of opposite polarities. If this instrumental artefact explained our findings at blue-on-yellow stimulation it would also affect blue-on-blue increment and decrement thresholds. In order to check this suggestion we measured thresholds for blue-on-blue increments and decrements with stimuli of selected sizes. The only

difference from the main experiment was the absence of the yellow background component.

2.3. Subjects

Two of the authors and 13 volunteers served as subjects. No one had abnormal ophthalmologic history or colour vision abnormality, tested by Ishihara cards. All subjects except for A.V. and M.Z., who were authors, were naïve concerning the aim of the study. A.V. was 62 years old, M.Z. was 45 years old, M.V. was 58 years old and all the remaining 12 subjects were between 23 and 28 years old.

The tenets of the Declaration of Helsinki were followed with regards to study subjects. Informed consent was obtained from each subject before enrolment in the study.

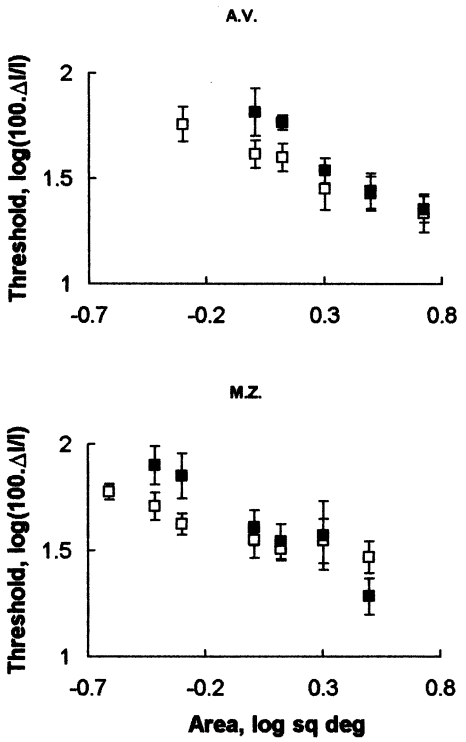

Fig. 6. Spatial summation of blue-on-yellow increments and decrements measured by an interleaved procedure as explained in the text. Open symbols — luminance increment; closed symbols — luminance decrement. Vertical bars — the 95% confidence intervals of the mean. Subjects A.V. and M.Z.

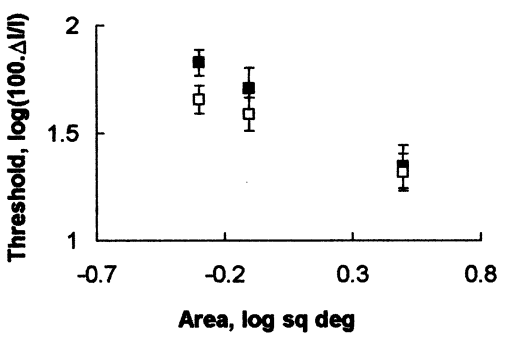

Fig. 7. Spatial summation of blue-on-yellow increments and decrements measured by an interleaved procedure. Open symbols — luminance increment; closed symbols — luminance decrement. Vertical bars — the 95% confidence intervals of the group means. Group averages across nine subjects.

3. Results

3.1. Spatial summation of blue-on-yellow and red-on-red luminance increments and decrements

The results of this experiment are shown in Figs. 4 and 5 as detection threshold versus stimulus area curves. Two subjects were tested with both blue-on-yellow and red-on-red stimuli and their data are shown in Fig. 4. In each graph of Fig. 4, the upper pair of curves (squares) is for the blue-on-yellow thresholds and the lower pair (triangles) is for the red-on-red thresholds. Open symbols represent the increment thresholds and closed symbols represent the decrement thresholds. It is seen that the thresholds were lower with red stimuli than with blue stimuli and that the enlargement of stimulus area reduced the detection threshold with any type of stimulus. It is also seen that the detection threshold of blue-on-yellow stimuli depended on the sign of the luminance change, positive (increment) or negative (decrement). The blue-on-yellow decrement threshold was higher than the increment threshold with stimuli of small size (a one-tailed t-test: $P < 0.001$ at 1° and $P < 0.001$ at 1.3° in diameter for subject A.V.; $P < 0.009$ at 0.9° and $P < 0.012$ at 1° in diameter for subject M.Z.). This combined effect of stimulus sign and size was so strong that one could diminish stimulus area up to a value at which the increment threshold could only be measured and not the decrement one (leftmost points in Fig. 4; upper pairs). On enlargement of the stimulus, the decrement threshold decreased to a larger amount than the increment threshold, the data suggesting that spatial summation was more effective with negative stimuli than with positive stimuli. On the contrary, no systematic differences between increment and decrement threshold were observed with red stimuli at 0.05 level.

The slope of the lines through the leftmost experimental points in Figs. 4 and 5 is -1. A slope of -1 of a threshold against stimulus area curve in double logarithmic co-ordinates corresponds to constancy of the threshold times area product, i.e. to complete spatial summation (Ricco's Law). The area of complete spatial summation was estimated by statistical evaluation of the test stimulus area up to which no significant deviation (at 0.05 level) from a line of the pre-selected slope of -1 and an adjustable constant was observed. The

procedure consisted in testing the goodness-of-fit of the line in several steps starting with the two points of smallest areas. The ratio of the component of variance, due to lack of fit, s_r, and the component of variance due to the experimental error, s_e, (see Appendix A) was calculated and tested against F-statistics. If the calculated F-ratio was smaller than the F-value needed to reject the adequacy of fitting at the 0.05 confidence level, the next point was added and a new line with a slope of -1 and adjustable free constant was fitted to the points. This procedure continued until a significant deviation occurred.

With red stimuli, complete summation was found up to a stimulus diameter of 0.4° (stimulus area of -0.9 log square degrees in Fig. 4) regardless of the stimulus sign. With blue stimuli, the area of complete summation was larger for light decrements than for light increments (see next paragraph for quantitative data).

In order to verify the last finding, four more subjects were tested. Their data are presented in Fig. 5. Again, the increase of stimulus area reduced more the threshold for luminance decrement than for luminance increment. With subject L.B. (Fig. 5) this effect was statistically insignificant at the 0.05 level as were the differences between increment and decrement threshold at any stimulus size, and the area of complete summation could not be measured at luminance increments. With this exception, the estimated diameter of the area of complete summation of blue-on-yellow stimuli varied between 0.6 and 0.9° for luminance increment (-0.5 and -0.2 log square degrees in Figs. 4 and 5) and between 0.8 and 2° for luminance decrement (stimulus area between -0.3 and 0.5 log square degrees in the same figures).

3.2. Blue-on-yellow increment and decrement thresholds measured simultaneously

Blue-on-yellow light increment was seen as a pale violet spot while light decrement was seen as saturated yellow spot on the yellow-plus-blue background. We assumed that the difference in hue might result in different criteria of stimulus detection particularly when the type of stimulus is constant within a block of trials. Such criteria might be more effective when stimulus type is known in advance. To avoid an anticipatory effect, experiments were performed during which luminance increment and decrement were intermixed randomly. Two staircases were run in parallel within a block of trials in order to measure both thresholds.

Eleven subjects took part in the experiment. Subjects A.V. and M.Z. were tested at five to seven stimulus sizes and Fig. 6 represents the mean thresholds obtained with them in four daily sessions. At the smallest stimulus sizes, the decrement threshold was higher than the increment threshold (a one-tailed t-test: $P<0.02$ at 1° and $P<0.002$ at 1.3° in diameter for subject A.V.; $P<0.005$ at 0.7° and $P<0.003$ at 0.8° for subject M.Z.). Increasing stimulus size reduced both thresholds but the threshold/area function was steeper with negative than positive flashes. Each of the remaining nine subjects was tested in a single daily experiment consisting of a training session and three sessions with stimuli of three sizes only. All nine subjects gave similar results and this allowed us to present their data as group averages (Fig. 7). Again, higher decrement than increment thresholds were measured with small stimuli. This relationship was found with each subject, the difference being significant for the group averages (a one-tailed t test: $P<0.001$ at 0.8° and $P<0.01$ at 1° in diameter). Increasing stimulus size reduced more the decrement threshold and the threshold/area function was again steeper for stimulus decrement thus suggesting a higher efficiency of spatial summation for negative than for positive flashes. In brief, the main finding of differences in spatial summation between blue-on-yellow luminance increment and decrement was confirmed.

3.3. Verification of the S-cone isolation

3.3.1. Spectral sensitivity

In order to verify the efficiency of the S-cone isolation, spectral sensitivity was measured and compared with the spectral sensitivity of Stiles π_1, π_4 and π_5 mechanisms. Three subjects took part in this experiment. The results are presented in Fig. 8. Note that the individual curves differed mostly at the shortest wavelengths and less for wavelengths above 550 nm which is a typical inter-individual variation (Pokorny, Smith & Lutze, 1987). Stiles curves are shifted vertically in order to fit the experimental data of Subject M.Z. whose sensitivity was the intermediate. As it is seen, the spectral sensitivity curves were nearly parallel to Stiles π_1 curve up to 500 nm. A comparison of the experimental data in Fig. 8 with the spectrum of the blue gun (Fig. 2) shows that, upon the chromatic adaptation in our experiments, the blue mechanism was the most sensitive to the test stimulus generated by the monitor blue gun (maximum intensity at 450 nm).

3.3.2. Blue stimulus detectability at different luminance levels of the yellow background component

Due to technical restrictions, the spectral sensitivity curves in the above control experiment were obtained in the absence of the blue background component. This component, when present, should adapt the S-cones and might reduce or even eliminate the S-cone

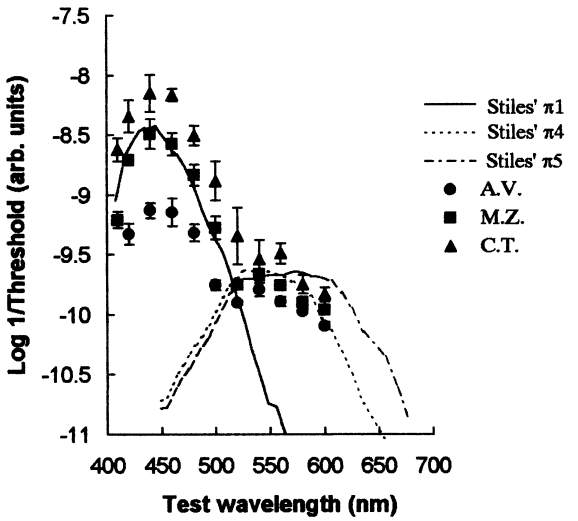

Fig. 8. Spectral sensitivity upon adaptation to the 300 cd/m^2 yellow background compared with Stiles π_1, π_4 and π_5 mechanisms. Experimental points — data of three subjects. Vertical bars — the 95% confidence intervals of the means. Stiles π_1, π_4 and π_5 curves are shifted vertically for a best fit by eye with the data of Subject M.Z.

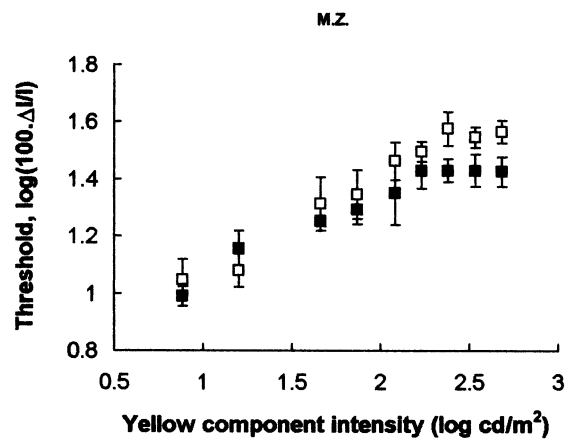

Fig. 9. Increment and decrement thresholds of blue-on-yellow stimuli as a function of the luminance of the yellow background component. The blue-component luminance was fixed at 3 cd/m^2. Open symbols — luminance increment; closed symbols — luminance decrement. Vertical bars — the 95% confidence intervals of the mean. Subject M.Z.

isolation. The second control experiment was aimed at verifying this assumption. The results of one subject are presented in Fig. 9. Both increment and decrement thresholds increased on increasing the yellow light intensity from 0 to 100–150 cd/m^2 and were nearly constant above that level. (Fig. 9). Similar data were also obtained with a second subject and, at the eccentricity of 4°, with two more subjects during pilot experiments aimed at selection of the yellow background intensity. Threshold constancy implies that the stimulus was detected by a mechanism independent of the yellow light, i.e. the 'blue' mechanism. This conclusion is further supported by the results of previous experiments performed with the same equipment (Vassilev, 1997): When small blue stimuli were presented on the yellow-plus-blue background, sensitivity was lower in the fovea than at 1–2° eccentricity where it was the highest. Such a dependency of sensitivity on retinal eccentricity is typical for the blue mechanism (Williams, MacLeod & Hayhoe, 1981).

The threshold values in Figs. 4–7 and 9 are presented as log percent modulation of the blue background component. In order to express the threshold values in S-cone contrast, we measured the radiances of the blue and yellow light sources at different wavelengths using the monochromator and radiometer. The values obtained were corrected according to the spectral calibration curves of the instruments and weighted by the S-cone spectral sensitivity 10° data and lens density spectrum of Stockman, Sharpe and Fach (1999). The estimated S-cone contrast was 97.9% of the blue stimulus contrast in Figs. 4–7 and 9, i.e. the contribution of the yellow background component to the S-cone excitation amounted at 2.1% only. This value should be corrected by the individual lens density spectra which might differ considerably (Pokorny et al., 1987) but no such data were available to us. However, the S-cone contrast reduction by the yellow background component should indeed be small as one could infer from the blue stimulus threshold independent of the intensity of the yellow light above 150 cd/m^2, as is illustrated in Fig. 9.

3.4. Blue-on-blue increment and decrement thresholds

In order to verify that the differences between increment and decrement thresholds were related to the S-cone signals rather than a test stimulus artefact, no yellow adapting background was used in this experiment. Detection threshold was measured with four subjects for one selected stimulus size only, the one at which the largest difference between increment and decrement thresholds was found for each subject in the main experiment. All other experimental conditions were identical with those of the main experiment. The data from the main experiment and from the present one are compared in Table 1. Contrary to the results obtained in the main experiment, no statistically significant difference between increment and decrement threshold was observed when they were measured with blue stimuli on blue background. This result allowed us to reject the assumption that the difference between blue increment and decrement thresholds in the main experiment was due to a stimulus artefacts such as light scatter from a lighter area over a dimmer area, a scatter that increases the area of positive test stimuli and decreases the area of negative stimuli. The effect of light scatter was probably negligible due to the large size of test stimuli.

Table 1
Blue-on-yellow and blue-on-blue increment (I) or decrement (D) thresholds

Subject	Test diameter (°)	Stimulus type	Adapting background[a]	
			Blue (3 cd/m^2)	Yellow (300 cd/m^2) + Blue (3 cd/m^2)
A.V.	0.8	I	1.16 ± 0.06	1.75 ± 0.08
		D	1.18 ± 0.04	>2
M.Z.	0.7	I	1.22 ± 0.07	1.71 ± 0.08
		D	1.21 ± 0.04	1.90 ± 0.09
C.T.	0.7	I	1.22 ± 0.05	1.61 ± 0.05
		D	1.23 ± 0.11	1.78 ± 0.06
N.Y.	1.6	I	0.64 ± 0.06	1.21 ± 0.08
		D	0.69 ± 0.06	1.05 ± 0.05

[a] Threshold ± S.D.

4. Discussion

The present study, in agreement with previous data (e.g. Brindley, 1970; King-Smith & Carden, 1976; Castano & Sperling, 1982; Haegestrom-Portnoy & Adams, 1988; Diaz, Jimenez, Hita & del Barco, 1998), has found that spatial summation upon selective stimulation of the S-cones takes place over larger areas than spatial summation when other cones, in the present case mainly the L-cones, are stimulated. In addition it was found that Ricco's area of summation of S-cone signals depends on the sign of luminance change, increment or decrement. The largest summation areas were observed for blue-on-yellow luminance decrement. At the retinal eccentricity tested, 12.5°, and on the background used, 300 cd/m^2 yellow light plus 3 cd/m^2 blue light, the diameter of Ricco's area was in the range between 0.6 and 0.9° for luminance increment and between 0.8 and 2° for luminance decrement. As far as we know, no other report on this asymmetry of spatial summation of blue-on-yellow signals has been presented so far. For most subjects, at small stimulus sizes, the decrement threshold was higher than the increment threshold. The size of the summation area did not depend on stimulus sign and was smaller when red-on-red stimuli were used. Blue-on-blue stimuli did not yield different increment and decrement thresholds.

An explanation of the asymmetry in spatial summation of blue-on-yellow luminance increment and decrement by properties of chromatic vision should be considered first. Blue luminance increment on yellow background evoked the sensation of pale violet (white according to some subjects) while the blue luminance decrement was perceived as dimmer yellow. Subjectively, hue changed more for increments than for decrements. Thus, the chromatic mechanisms might be more important than the luminance mechanisms in detecting blue-on-yellow luminance increment than decrement. In view of the lower spatial resolution of the chromatic mechanisms in comparison with the luminance ones (e.g. Brindley, 1970; King-Smith & Carden, 1976; Mullen, 1990) one should expect a larger summation effect for blue-on-yellow luminance increment than decrement. The results were the opposite and do not support such an assumption.

A tempting interpretation of the present findings is in terms of the S-cone ON–OFF asymmetry pointed out in Section 1 towards more ON type pathways than OFF pathways receiving S-cone input (Malpeli & Schiller, 1978; De Monasterio, 1979; Lee, 1996). Previous studies (Smith et al., 1989; De Marco et al., 1994; Schwartz, 1996) tested the hypothesis that the S-cone ON-OFF asymmetry should result in a higher decrement threshold than increment threshold. With one exception, to be discussed later, all these studies used large test stimuli. Those of our data that were obtained with large stimuli too, confirm their findings: sensitivity to luminance decrements was not inferior to sensitivity to luminance increments. Contrary to the expected ON–OFF sensitivity relationship, the decrement threshold was even lower with some subjects when the test stimulus was large, a finding that can be also seen in the data published by Schwartz (1996).

The present study tested another hypothesis put forward in Section 1, namely that the extent of spatial summation is inversely related to the density of S-cone pathways. It was based on the following facts from visual physiology, morphology and psychophysics, pointed out in Section 1: (a) lower encounter rate of blue-OFF than of blue-ON cells in monkey; (b) inverse relationship between density and dendritic field size of the known types of retinal ganglion cells; and (c) inverse relationship between ganglion cell density and extent of spatial summation. If the lower encounter rate of blue-OFF cells is not due to recording electrode bias (Valberg et al., 1986) and if dendritic field size determines spatial signal convergence, summation of blue light decrements should occur over larger areas than

summation of blue light increments. All but one of our 15 subjects provided data supporting this hypothesis. Expressed in square degrees, the area of complete spatial summation (Ricco's area) for blue-on-yellow luminance decrements, as measured with five subjects, was typically twice as large as the area of summation for luminance increments. The data of nine subjects tested at a few selected stimulus sizes only do not allow to estimate the area of summation yet they do show a higher efficiency of stimulus enlargement for blue luminance decrements than for increments.

The dendritic fields of ganglion cells at distances larger than 7° from the fovea provide the major site of spatial convergence (Lee, 1996). It is, therefore, tempting to compare Ricco's areas obtained in the present experiments with the existing data about the dendritic field size of ganglion cells in the human retina. There are, however, limitations of such a comparison. Ricco's area for both achromatic (Barlow, 1958) and chromatic (Saudargene & Bertulis, 1980) vision depends on the level of retinal illumination and is, therefore, determined by factors additional to the dendritic field size. Yet, a constant ratio between Ricco's area, measured at a fixed background luminance level, and dendritic field size might be expected. In view of the presence of morphological data about the dependence of dendritic field size of different retinal ganglion cell types on retinal eccentricity (Dacey & Petersen, 1992; Dacey, 1993), further experiments with stimuli presented at a range of retinal locations might provide data necessary to verify the assumed relationship between Ricco's area and retinal morphology and contribute to the identification of the type of cells mediating stimulus detection.

In one of their psychophysical experiments on rhesus monkeys, Smith et al. (1989) varied stimulus size and compared control thresholds with thresholds obtained after application of APB. A 3000 td white adapting field was used and the predominant test stimulus wavelength was 530 or 450 nm. Stimulus retinal location was not specified by the authors. APB elevated the threshold by the same amount at both wavelengths and at all stimulus sizes. As far as APB blocks selectively the ON pathway, the size-independent threshold elevation implies no spatial differences between the ON and OFF pathways. Unfortunately, the authors' aim being to find out the asymptotic level in the threshold/stimulus area curve, the range of stimulus sizes included stimuli larger than Ricco's area (checked by us using the data in their Fig. 7) and the adapting conditions were too different from those at which they proved the S-cone isolation.

One might argue that stimulus duration (100 ms) was long enough to elicit both ON and OFF responses. Calculations based on the temporal impulse response to white stimuli show that both onset and offset responses are nearly equal in amplitude at the duration of 100 ms (Krauskopf, 1980). This should not be, however, the case in the present experiments with blue-on-yellow stimuli. Krauskopf based his calculations on data obtained with white stimuli at an adaptation level of 7×10^4 trolands and his own data (Krauskopf & Mollon, 1971) have shown that the short-wavelength mechanism has a particularly large critical duration. The adaptation level in the present experiments was 5×10^3 trolands or less depending on the subject's pupil size. Moreover, the blue component, the only one which could excite the S-cones, provided an adaptation level of 60 trolands or less than that. The lower adaptation state in the present experiments should result in slower response characteristics. Indeed De Lange curves obtained with the same equipment and stimulus diameter of 1° (unpublished data) show low-pass characteristics with a sharp decline above 4–6 Hz of the blue mechanism and confirm the previously reported slowness of the S-cone pathways (see Gouras & MacKay, 1990, Stockman et al., 1991; Yeh, Lee & Kremers, 1995, for experimental conditions yielding a fast response to S-cone stimulation). We assume that, under the present experimental conditions, 100 ms stimulus duration of the blue-on-yellow stimuli was short enough to preferentially activate the S-cone ON or OFF pathways, depending on stimulus sign. Having in mind the known interaction between stimulus size and response temporal characteristics (Barlow, 1958), the predominance of the ON-response to luminance increments or the predominance of the OFF-response at luminance decrements should be particularly pronounced with small stimuli.

If the current interpretation of our data is correct, the present findings might have some practical implications. The most obvious among them is the possibility to evaluate the function of the least numerous visual pathway when a disease that might affect retinal ganglion cells such as glaucoma is suspected. The less dense a cellular type is, the lower the redundancy is and the more probable is a functional deficit in an early stage of the disease. Retinal ganglion cells with an S-cone OFF input are the least dense (De Monasterio, 1979; Lee, 1996). Looking for a functional deficit among the least numerous type of ganglion cells might be advantageous for the sake of early diagnosis.

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Appendix A

The component of variance, due to lack of fit, s_r^2, and the component of variance due to the experimental error, s_e^2, were calculated as follows (Bard, 1974):

$$s_r^2 = \sum_i^N n_i(y_i - y_i*)^2/k_1; \quad k_1 = N - p$$

$$s_e^2 = \sum_i^N \sum_j^{n_i} n_i(y_{ij} - y_i)^2/k_2; \quad k_2 = \sum_i^N n_i - N$$

where N is the number of data points, n_i is the number of repeated measurements at the ith data point, y_{ij} is an individual measurement, y_i is the mean value of each group of repeated measurements, y_i^* is the calculated value from the line with given parameters, p is the number of line parameters, k_1 and k_2 are degrees of freedom.

The s_r^2/s_e^2 ratio was calculated and tested against F-statistics. It was accepted that the line with given parameters adequately fits the data points if $s_r^2/s_e^2 < F_{1-\alpha}(k_1, k_2)$ for $\alpha < 0.05$.

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